Conventon Application for a Patent

We

ELI LILLY AND COMPANY

of

307 East McCarty Street, City of Indianapolis,
State of Indiana, United States of America

hereby apply for the grant of a Patent for an invention entitled

"ANALGESIC COMPOUNDS"

which is described in the accompanying complete specification.
This application is a Convention Application and is based on the
application numbered 838,516
for a patent or similar protection made in United States of America

on 3rd October, 1977

Our address for service is:
Care: SPRUSON & FERGUSON
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AUSTRALIA.

Dated this FOURTEENTH day of SEPTEMBER, 1978

ELI LILLY AND COMPANY

By: ____________________________
    Signature of Applicant

To: The Commissioner of Patents

Registered Patent Attorney
DECLARATION IN SUPPORT OF CONVENTION APPLICATION
FOR A PATENT OR PATENT OF ADDITION

(The declaration shall be made by the applicant, or, if the applicant is a body corporate, by a person authorized by the body corporate to make the declaration on its behalf.)

In support of the Convention Application made for a patent or patent of addition for an invention entitled

"ANALGESIC COMPOUNDS"

I, -We-,

Everett Foy Smith
3926 Glenview Drive
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do solemnly and sincerely declare as follows:—

1. (a) I am the applicant for the patent

or (b) I am authorized by Eli Lilly and Company, a corporation of the State of Indiana, United States of America

the applicant for the patent of addition to make this declaration on its behalf.

2. The basic application as defined by Section 141 of the Act was made in the United States of America on the 3rd October 1977

by Edward Lee Smithwick, Jr., Robert Curtis Arthur Frederickson and Robert Theodore Shuman

and the United States of America on the

by Edward Lee Smithwick, Jr., of 8250 Shadow Circle, Indianapolis, Indiana, United States of America; Robert Curtis Arthur Frederickson, 4225 Marrison Place, Indianapolis, Indiana, United States of America and Robert Theodore Shuman, 4155 Rolling Trails Greenwood, Indiana, United States of America

are the actual inventor(s) of the invention and the facts upon which the applicant are entitled to make the application are as follows:—

The applicant company is assignee of the inventor(s)

4. The basic application referred to in paragraph 2 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application.

Indianapolis, 5th day of September 1978.

ELI LILLY AND COMPANY

BY: Everet Foy Smith
Compounds exhibit analgesic activity.

Claim

1. A compound of the general formula

\[
\begin{align*}
&\text{(L) (D) (I)} \\
&\text{R} \quad \text{O} \\
&\text{N-CH-CONH-CH-C-NH-CH-C-N-C-Z} \\
&\text{R}_1 \text{CH}_2 \text{R}_2 \text{CH}_2 \text{R}_3 \text{CH}_2 \text{R}_4 \text{CH}_2 \\
&\text{OY}
\end{align*}
\]

and pharmaceutically acceptable non-toxic acid addition salts thereof, in which L and D, when applicable, define the chirality;

- \( R_1 \) and \( R_2 \) independently are hydrogen or \( C_1 - C_3 \) primary alkyl;
- \( R_3 \) is \( C_1 - C_4 \) primary or secondary alkyl or \( -CH_2CH_2S-CH_3 \);
$R_4$ is hydrogen or C$_1$-C$_3$ primary alkyl; 
$R_5$ is hydrogen or C$_1$-C$_3$ primary alkyl; 
$Y$ is hydrogen or acetyl; and 
$O$

is -C=NH$_2$, -$\text{CH}_2$OH, or -CN; subject to the

limitation that one of $R_4$ and $R_5$ is C$_1$-C$_3$ primary

alkyl and the other is hydrogen.
Complete Specification for the invention entitled:

"ANALGESIC COMPOUNDS"

The following statement is a full description of this invention, including the best method of performing it known to me/us:
ANALGESIC COMPOUNDS
This invention relates to a novel class of compounds which exhibit analgesic activity upon parenteral administration.

Recently, endogenous substances having morphine-like properties have been extracted from mammalian brain or cerebral spinal fluid (csf). These substances, named enkephalin, have been identified by Hughes et al., Nature, 258, 577 (1975) as pentapeptides having the following sequences:

H-Tyr-Gly-Gly-Phe-Met-OH
H-Tyr-Gly-Gly-Phe-Leu-OH.

These compounds are referred to as methionine-enkephalin and leucine-enkephalin, respectively.

Although these compounds have been shown to exhibit analgesic activity in mice upon administration intracerebroventricularly [Buscher et al., Nature, 261, 423 (1976)], they are practically devoid of any useful analgesic activity when administered parenterally.
A novel class of compounds has now been discovered. These compounds exhibit significant and demonstrable analgesic activity when administered systemically. It is to this class of compounds that this invention is directed.

Thus, this invention relates to a class of compounds having the general formula

\[
\begin{align*}
\text{CH} - \text{CH} - \text{CH} - \text{NH} - \text{C} - \text{NH} - \text{C} - \text{NH} - \text{CH} - \text{C} - \text{N} - \text{C} - \text{Z} \\
\text{O} - \text{Y}
\end{align*}
\]

and pharmaceutically acceptable non-toxic acid addition salts thereof, in which L and D, when applicable, define the chirality;

- \( R_1 \) and \( R_2 \) independently are hydrogen or \( C_1-C_3 \) primary alkyl;
- \( R_3 \) is \( C_1-C_4 \) primary or secondary alkyl or \(-\text{CH}_2\text{CH}_2\text{S-CH}_3\);
- \( R_4 \) is hydrogen or \( C_1-C_3 \) primary alkyl;
- \( R_5 \) is hydrogen or \( C_1-C_3 \) primary alkyl;
- \( Y \) is hydrogen or acetyl; and
Z is \(-\text{C-NH}_2\), \(-\text{CH}_2\text{OH}\), or \(-\text{CN}\); subject to the limitation that one of \(R_4\) and \(R_5\) is \(\text{C}_1-\text{C}_3\) primary alkyl and the other is hydrogen.

The compounds of formula (I) are prepared by cleaving the blocking agents from the correspondingly protected compound of formula (I) with a substantially dry acid medium.

Pharmaceutically acceptable non-toxic acid addition salts include the organic and inorganic acid addition salts, for example, those prepared from acids such as hydrochloric, sulfuric, sulfonic, tartaric, fumaric, hydrobromic, glycolic, citric, maleic, phosphoric, succinic, acetic, nitric, benzoic, ascorbic, \(p\)-toluenesulfonic, benzenesulfonic, naphthalenesulfonic, and propionic. Preferably, the acid addition salts are those prepared from hydrochloric acid, acetic acid, or succinic acid. Any of the above salts are prepared by conventional methods.

As will be noted from the definition of the various substituents which appear in formula (I), the compounds which are defined by this structure are the primary amide, primary alcohol, or nitrile derivatives of specifically defined tetrapeptides.

The stereoconfiguration of the compounds of formula (I) is an essential feature thereof. For the sake of convenience, the amino acid residues of the modified tetrapeptides of formula (I) are numbered sequentially beginning with the residue at the terminal amino function. The chirality of the amino acid residues, reading from Position 1 through
Position 3, is L, D, and none. The residue in Position 3 is a glycine moiety, and, thus, no chirality as to this residue exists. As to Position 4 (the C-terminal position) which is a primary amide, a primary alcohol, or a nitrile, its chirality is defined as that which is consistent with the corresponding putative L-amino acid residue.

The groups R₁, R₂, R₄, and R₅ as used herein are defined to include the group "C₁-C₃ primary alkyl". By the term "C₁-C₃ primary alkyl" is intended methyl, ethyl, and n-propyl.

The group R₃ appearing in the above structural formula is defined to include the group "C₁-C₄ primary or secondary alkyl". By the term "C₁-C₄ primary or secondary alkyl" is meant methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, and sec-butyl.

With respect to the particular residues in each of the positions of the modified tetrapeptides of formula (I), the following considerations prevail:


This position represents the amino-terminal portion of the peptide. The residue is that which results from L-tyrosine or L-(O-acetvl)tyrosine. In either instance, the residue can be N-unsubstituted, in which case both R₁ and R₂ are hydrogen. Moreover, the residue can be substituted by one or two C₁-C₃ primary alkyl groups, in which case R₁ and/or R₂ is C₁-C₃ primary alkyl. Specific illustrations of C₁-C₃ primary alkyl substitution include N-methyl-, N-ethyl-, N-n-propyl-, N,N-dimethyl, N,N-diethyl, N,N-di-N-propyl, N-methyl-N-ethyl, N-methyl-
N-n-propyl, and N-ethyl-N-n-propyl. Preferably, the tyrosyl or O-acetyltyrosyl residue which is present in Position 1 of the peptide of formula (I) is N-unsubstituted. Furthermore, it is preferred that the residue is tyrosyl.

(B). Position 2.

The amino acid residue which is present in the second position of the peptide of formula (I) must be the D stereoisomer and is any of several amino acid residues. These include residues derived from D-alanine (Ala) ($R_3$ is methyl), D-$\alpha$-amino-butyric acid (Abu) ($R_3$ is ethyl), D-norvaline (Nva) ($R_3$ is n-propyl), D-valine (Val) ($R_3$ is isopropyl), D-norleucine (Nle) ($R_3$ is n-butyl), D-leucine (Leu) ($R_3$ is isobutyl), D-iso-leucine (Ile) ($R_3$ is sec-butyl), and D-methionine (Met) ($R_3$ is $-\text{CH}_2\text{CH}_2\text{S-CH}_3$). Preferably, the residue is that derived from D-alanine.

(C). Position 3.

The amino acid residue present in this position is that derived from glycine (Gly).


The residue present in the C-terminal position is that derived from L-phenylalanine (Phe) or the primary alcohol or nitrile derivative thereof. The residue can be a primary amide (Phe-$\text{NH}_2$) ($Z$ is $-\text{C-NH}_2$), a primary alcohol (Phe-$\text{A}$) ($Z$ is $-\text{CH}_2\text{OH}$), or a nitrile (Phe-$\text{CN}$) ($Z$ is $\text{-CN}$). A preferred class of compounds is that in which $Z$ is $-\text{C-NH}_2$. 

The residue can be either unsubstituted or substituted at the amino nitrogen \((R_4)\). In the event that the residue is N-substituted, it is N-methyl, N-ethyl, or N-n-propyl. In addition, in the event that the residue is unsubstituted at the amino nitrogen, it must be substituted at the \(\alpha\)-carbon \((R_5)\). In such instances, \(R_5\) is methyl, ethyl, or n-propyl. The only limitation is that one of \(R_4\) and \(R_5\) is \(C_1-C_3\) primary alkyl and the other is hydrogen.

More preferably, the \(C_1-C_3\) primary alkyl group is methyl. Thus, highly preferred compounds are those in which \(R_4\) or \(R_5\) is methyl, and most preferably, those in which \(R_4\) is methyl.

In this specification, the following abbreviations, most of which are well known and are commonly used in the art, are employed:

- Abu - \(\alpha\)-aminobutyric acid
- Ala - alanine
- Cys - cysteine
- Gly - glycine
- Hse - homoserine
- Ile - isoleucine
- Leu - leucine
- Met - methionine
- Nle - norleucine
- Nva - norvaline
- Phe - phenylalanine
- Phe-NH$_2$ - phenylalanine amide
- Phe-A - primary alcohol derivative of phenylalanine
- Phe-CN - nitrile derivative of phenylalanine
Ser - serine
Tyr - tyrosine
Val - valine
Ac - acetyl
Me - methyl
Et - ethyl
Ip - isopropyl
Pr - n-propyl
Bu - n-butyl
10
i-Bu - isobutyl
t-Bu - t-butyl
s-Bu - sec-butyl
BOC - t-butyloxycarbonyl
Bzl - benzyl
15
DCC - N,N'-dicyclohexylcarbodiimide
HBT - 1-hydroxybenzotriazole
DMF - N,N-dimethylformamide
TFA - trifluoroacetic acid
THF - tetrahydrofuran
20
DEAE - diethylaminoethyl
DCHA - dicyclohexylamine

Examples of typical compounds of formula (I) include the following:

H-L-Tyr-D-Ala-Gly-L-(N-Et)Phe-NH_{2};
H-L-Tyr-D-Ala-Gly-L-(N-Pr)Phe-NH_{2};
H-L-Tyr-D-Ala-Gly-L-(N-Pr)Phe-A;
H-L-Tyr-D-Ala-Gly-L-(α-Me)Phe-NH_{2};
H-L-Tyr-D-Ala-Gly-L-(N-Me)Phe-NH_{2};
H-L-Tyr-D-Ala-Gly-L-(α-Et)Phe-NH_{2};
H-L-Tyr-D-Ala-Gly-L-(N-Me)Phe-CN;
H-L-Tyr-D-Ala-Gly-L-(N-Me)Phe-A;
H-L-Tyr-D-Met-Gly-L-(N-Et) Phe-NH₂;
H-L-Tyr-D-Met-Gly-L-(N-Me) Phe-NH₂;
H-L-Tyr-D-Met-Gly-L-(N-Me) Phe-A;
H-L-Tyr-D-Met-Gly-L-(N-Et) Phe-CN;

H-L-Tyr-D-Met-Gly-L-(α-Et) Phe-NH₂;
H-L-Tyr-D-Met-Gly-L-(α-Pr) Phe-NH₂;
H-L-Tyr(Dc)-D-Ala-Gly-L-(N-Me) Phe-NH₂;
H-L-Tyr(Dc)-D-Nle-Gly-L-(α-Me) Phe-NH₂;
(N-Et)-L-Tyr-D-Abu-Gly-L-(N-Et) Phe-NH₂;
(N,N-di-Pr)-L-Tyr-D-Val-Gly-L-(α-Me) Phe-NH₂;
(N-Pr)-L-Tyr-D-Leu-Gly-L-(α-Et) Phe-NH₂;
(N,N-di-Et)-L-Tyr-D-Met-Gly-L-(α-Pr) Phe-NH₂;
(N-Me,N-Et)-L-Tyr(Dc)-D-Nle-Gly-L-(α-Me) Phe-NH₂;
(N,N-di-Me)-L-Tyr(Dc)-D-Ile-Gly-L-(α-Et) Phe-NH₂;
(N-Me)-L-Tyr(Dc)-D-Leu-Gly-L-(α-Me) Phe-CN;
(N-Me)-L-Tyr(Dc)-D-Nva-Gly-L-(α-Pr) Phe-A;
(N,N-d ME)-L-Tyr-D-Ala-Gly-L-(α-Me) Phe-NH₂;
(N,N-di-Me)-L-Tyr-D-Ala-Gly-L-(α-Pr) Phe-NH₂;
H-L-Tyr-D-Ala-Gly-L-(α-Me) Phe-CN;
H-L-Tyr-D-Ala-Gly-L-(α-Me) Phe-A;
H-L-Tyr-D-Ala-Gly-L-(α-Et) Phe-A;
H-L-Tyr-D-Ala-Gly-L-(α-Pr) Phe-NH₂;
(N,N-di-Me)-L-Tyr-D-Ala-Gly-L-(α-Pr) Phe-CN;
(N,N-di-Me)-L-Tyr-D-Ala-Gly-L-(N-Me) Phe-NH₂;
(N,N-di-Et)-L-Tyr-D-Ala-Gly-L-(N-Et) Phe-CN;
(N-Me)-L-Tyr-D-Ala-Gly-L-(N-Me) Phe-A;
(N-Me)-L-Tyr-D-Ala-Gly-L-(N-Me) Phe-CN;
(N,N-di-Me)-L-Tyr-D-Ala-Gly-L-(N-Me) Phe-NH₂;
(N,N-di-Me)-L-Tyr-D-Ala-Gly-L-(N-Me) Phe-NH₂;
(N,N-di-Me)-L-Tyr-D-Ala-Gly-L-(N-Pr) Phe-NH₂;
(N-Me)-L-Tyr-D-Ala-Gly-L-(α-Et) Phe-NH₂;
(N,N-Di-Me) -L-Tyr(Ac) -D-Ala-Gly-L-(N-Et) Phe-CN;
(N,N-Di-Pr) -L-Tyr(Ac) -D-Met-Gly-L-(N-Me) Phe-A;
(N-Et) -L-Tyr(Ac) -D-Met-Gly-L-(N-Pr) Phe-NH₂; and
(N-Me) -L-Tyr(Ac) -D-Met-Gly-L-(α-Me) Phe-NH.

The compounds of formula (I) are prepared by routine methods for peptide synthesis. It is possible, during the synthesis of certain of the compounds of formula (I), that partial racemization can occur. However, the extent of racemization, should such occur, is not sufficient to seriously alter the analgesic activity of the compounds of formula (I).

The methods for preparing the compounds of formula (I) involve the coupling of amino acids or peptide fragments by reaction of the carboxyl function of one with the amino function of another to produce an amide linkage. In order to effectively achieve coupling, it is desirable, first, that all reactive functionalities not participating directly in the reaction be inactivated by the use of appropriate blocking groups, and, secondly, that the carboxyl function which is to be coupled be appropriately activated to permit coupling to proceed. All of this involves a careful selection of both reaction sequence and reaction conditions as well as utilization of specific blocking groups so that the desired peptide product will be realized. Each of the amino acids which is employed to produce the compounds of formula (I) and which has the particularly selected protecting groups and/or activating functionalities is prepared by employing techniques well recognized in the peptide art.
Selected combinations of blocking groups are employed at each point of the total synthesis of the compounds of formula (I). These particular combinations have been found to function most smoothly. Other combinations would operate in the synthesis of the compounds of formula (I), although, perhaps, with a lesser degree of success. Thus, for example, benzyloxy carbonyl (CBz), t-butyloxy carbonyl (BOC), t-amyloxy carbonyl (AOC), p-methoxybenzyloxy carbonyl (MBOC), adamantyloxy carbonyl (AdOC), and isobornyloxy carbonyl can be variously employed as amino blocking groups in the synthesis of the compounds of formula (I). Furthermore, benzyl (Bzl) generally is employed as the hydroxy-protecting group for the tyrosyl residue even though others, such as p-nitrobenzyl (PNB), p-methoxybenzyl (PMB), could well be employed.

The carboxyl blocking groups used in preparing the compounds of formula (I) can be any of the typical ester-forming groups, including, for example, methyl, ethyl, benzyl, p-nitrobenzyl, p-methoxybenzyl, and 2,2,2-trichloroethyl.

Coupling of the suitably protected N-blocked amino acid or peptide fragment with a suitably protected carboxy-blocked amino acid or peptide fragment in preparation of the compounds of formula (I) consists of rendering the free carboxyl function of the amino acid or peptide fragment active to the coupling reaction. This can be accomplished using any of several well recognized techniques. One such activation technique involves conversion of the...
carboxyl function to a mixed anhydride. The free carboxyl function is activated by reaction with another acid, typically a derivative of carbonic acid, such as an acid chloride thereof. Examples of acid chlorides used to form mixed anhydrides are ethyl chloroformate, phenyl chloroformate, sec-butyl chloroformate, isobutyl chloroformate, and pivaloyl chloride. Preferably, isobutyl chloroformate is employed.

Another method of activating the carboxyl function for the purpose of carrying out the coupling reaction is by conversion to its active ester derivative. Such active esters include, for example, a 2,4,5-trichlorophenyl ester, a pentachlorophenyl ester, and a p-nitrophenyl ester. Another coupling method available for use is the well-recognized azide coupling method.

The preferred coupling method in preparation of the compounds of formula (I) involves the use of N,N'-dicyclohexylcarbodiimide (DCC) to activate the free carboxyl function thereby permitting coupling to proceed. This activation and coupling technique is carried out employing an equimolar quantity of DCC relative to the amino acid or peptide fragment and is carried out in the presence of an equimolar quantity of 1-hydroxybenzotriazole (HBT). The presence of HBT suppresses undesirable side reactions including the possibility of racemization.

Cleavage of selected blocking groups is necessary at particular points in the synthetic sequence employed in preparation of the compounds of formula (I). A chemist of ordinary skill in the art
of peptide synthesis can readily select from representative protecting groups those groups which are compatible in the sense that selective cleavage of the product can be accomplished permitting removal of one or more but less than all of the protecting groups present on the amino acid or peptide fragment. These techniques are well recognized in the peptide art. A fuller discussion of the techniques which are available for selective cleavage is provided in the literature in Schröder and Lübke, *The Peptides*, Volume I, Academic Press, New York, (1965), and especially in the Table provided at pages 72-75 thereof.

Cleavage of carboxyl protecting groups can be accomplished by alkaline saponification. Relatively strong alkaline conditions, typically using an alkali metal hydroxide, such as sodium hydroxide, potassium hydroxide, and lithium hydroxide, are generally employed to deesterify the protected carboxyl. The reaction conditions under which saponification is accomplished are well recognized in the art. The carboxyl blocking groups also can be removed by catalytic hydrogenolysis including, for example, hydrogenolysis in the presence of a catalyst such as palladium-on-carbon. Furthermore, in those instances in which the carboxyl blocking group is p-nitrobenzyl or 2,2,2-trichloroethyl, deblocking can be accomplished by reduction in the presence of zinc and hydrochloric acid.

The amino blocking groups are cleaved by treating the protected amino acid or peptide with an acid such as formic acid, trifluoroacetic acid
(TFA), p-toluenesulfonic acid (TSA), benzenesulfonic acid (BSA), and naphthalenesulfonic acid, to form the respective acid addition salt product. Cleavage of the amino blocking group also can be accomplished by treating the blocked amino acid or peptide with a mixture of HBr or HCl and acetic acid to produce the corresponding hydrobromide or hydrochloride acid addition salt. The particular method or reagent which is employed will depend upon the chemical or physical characteristics of the materials involved in the specific deblocking reaction. It has been discovered, in those instances in which the group R₄ is other than hydrogen and a peptide containing at least three amino acid residues is to be deblocked, that it is highly preferred that the peptide be deblocked with trifluoroacetic acid or formic acid to produce the corresponding acid addition salt. The salt can be converted to a more pharmaceutically acceptable form by treatment with a suitable ion exchange resin, such as DEAE Sephadex A25, and Amberlyst A27.

The hydroxy-protecting group present on the tyrosyl residue can be retained on the peptide throughout the sequence of its preparation, being removed during the final synthetic step in conjunction with cleavage of the amino blocking group. However, depending upon the conditions employed for removal of the carboxyl blocking group, it may be removed earlier in the preparative sequence. When the carboxyl group is cleaved by alkaline saponification, the hydroxy-protecting group is retained; however, when catalytic hydrogenolysis is employed for removal
of the carboxyl protecting group, the hydroxy protecting group also is cleaved. The latter situation does not represent a serious problem since preparation of the compounds of formula (I) can be accomplished in the presence of a tyrosyl residue having a free hydroxyl group.

A preferred specific method for preparing the compounds of formula (I) involves coupling a separately prepared N-terminal tripeptide with a separately prepared C-terminal phenylalanyl amide (Z is -C-NH$_2$) or its corresponding alcohol (Z is -CH$_2$OH) or nitrile (Z is -CN) followed by appropriate deblocking of any remaining blocked moieties. Alternatively, the separately prepared C-terminal phenylalanyl compound which is reacted with the N-terminal tripeptide can be structured so as to contain a group which represents a precursor to any of the amide, alcohol, or nitrile moieties. The general sequence, illustrating preparation of a tetrapeptide of formula (I), can be depicted as follows. In the sequence, the symbol AA represents the amino acid residue, and the appended number represents the position of the amino acid in the ultimate peptide product sequence.
A. Preparation of the tripeptide segment.

\[
\begin{align*}
\text{BOC-L-Tyr-OH} & \quad + \quad \text{H-D-(AA)z-OBzI} \\
\text{DCC} & \quad \text{HBT}
\end{align*}
\]

\[
\begin{align*}
\text{BOC-L-Tyr-D-(AA)z-OBzI} & \quad \text{OH} \\
\text{OBzI} & \quad \text{H-Gly-OBzI} \\
\text{DCC} & \quad \text{HBT}
\end{align*}
\]

\[
\begin{align*}
\text{BOC-L-Tyr-D-(AA)z-Gly-OBzI} & \quad \text{H2Pd/C} \\
\text{OBzI} & \quad \text{NaOH} \\
\text{THF/H2O}
\end{align*}
\]
B. Coupling of tripeptide and terminal phenylalanyl moiety.

In the above reaction, Ph represents phenyl and Q is -C-NH₂, -CH₂OH, -CN, -COCH₃, -C-OCH₂Ph, and other like groups.

When Q is -C-OCH₃, -C-OCH₂Ph and other like ester groups, it can be converted, after coupling, to -CH₂OH by treatment with NaBH₄. This reduction technique is described in Yamamura et al., U.S. Patent No. 3,700,651. When Q represents a benzyl ester or other ester comprising a group which is readily removable by hydrogenolysis, it can be converted to the free acid by hydrogenolysis in the presence of palladium-on-carbon. The free acid is convertible to the amide by treatment with ammonia in the presence of DCC and HBT.
The amide moiety can be dehydrated to the nitrile by treatment with p-toluenesulfonyl chloride and pyridine in accordance with the method described in Yamada et al., Bull. of the Chem. Soc. of Japan, 50, 1088-1093 (1977).

In preparing the compounds of formula (I) by the aforedescribed sequence, it is highly preferred to employ, as C-terminal reactant, a compound which contains the group Z of the intended final product. Once the intended modified tetrapeptide having the C-terminal group has been prepared, the O-protecting group on the tyrosyl (if such is present) can be removed by hydrogenolysis and the N-BOC protecting group by treatment with trifluoroacetic acid.

The above represents only one sequence for preparing compounds of formula (I). Other sequences are available. Another method which can be employed involves the step-wise, sequential addition of single amino acids or derivatives thereof in construction of the peptide chain beginning with the carboxamide, alcohol, or nitrile terminal moiety. Reaction techniques such as those described above would be employed in this as well as any other contemplated preparative sequence.

A further method for preparing compounds of formula (I) is solid phase synthesis. In this method the C-terminal residue is attached to a suitable polymeric support, and the peptide is extended one residue at a time until the desired peptide, still attached to the polymer support, is synthesized. The peptide then is removed from the
support by use of a suitable deblocking reagent. For example, the C-terminal moiety, protected at the α-amino by a t-butyloxy carbonyl group, is coupled to a benzhydrylamine polymer by DCC activation. The N-BOC group is removed by reaction of the polymer attached residue with trifluoroacetic acid in methylene chloride. The resulting salt is neutralized with a suitable tertiary amine, and the sequence repeated for addition of each successive amino acid. Upon completion of preparation of the intended peptide sequence, the blocked peptide is removed from the polymeric support by treatment with HF at 0°C. The product then can be purified by chromatography. The specific conditions of the synthesis, e.g., reaction times, reaction temperatures, wash times, reagents, protecting groups, and the like, are such as one of ordinary skill in the art of solid phase peptide synthesis would well recognize.

Cleavage of the peptide from the polymeric support achieves removal of all blocking groups with formation of the tetrapeptide intermediate. Since it is highly desirable to retain such protecting groups in conversion of the product to the nitrile compound, solid phase synthesis is not a desirable method for preparing compounds of formula (I) in which Z is -CN.

In certain of the compounds of formula (I), one or more of the groups R_1, R_2, and R_4 are C_1-C_3 primary alkyl. In those instances, the appropriate N-substituted amino acid is employed in the preparative sequence. Any of the N-monosubstituted amino acids can be prepared by the same sequence which is
depicted as follows using an N-protected amino acid as starting material:

\[
\begin{align*}
\text{H} & \quad \text{KH} \\
\text{BOC-N-(AA)-COOH} & \quad \overset{18\text{-crown-6 ether}}{\text{THF}} \quad \overset{\text{DMF}}{\text{DMF}} \\
\text{BOC-N-(AA)-COO}^- & \quad \text{K}^+ \\
\text{C-C primary} & \quad \text{alkyl iodide (} R_a \text{)} \\
\text{R}_a & \\
\text{BOC-N-(AA)-COOH}
\end{align*}
\]

As the above sequence indicates, the N\textsuperscript{\textalpha}-protected amino acid first is treated with potassium hydride in the presence of a suitable crown ether to generate the dianion. The intermediate then is treated with the appropriate alkyl iodide to obtain the desired N-substituted amino acid.

It will be apparent to those of ordinary skill in the art of peptide synthesis that racemization at the \(\alpha\)-carbon can occur under strongly alkaline conditions such as those employed in the above alkylation procedure. The degree of racemization may vary depending upon the particular amino compound which is involved. Racemization can be minimized by using excess alkylating agent and by keeping the reaction time as short as possible. Nevertheless, even in the event that excessive racemization does occur, the product can be purified by recrystallization as the salt of a suitable chiral amine, for example, as the salt of d(+)-\(\alpha\)-phenylethylamine.
The resulting amino acid in which \( R_4 \) is \( C_1-C_3 \) primary alkyl can be converted to its corresponding amide, alcohol, or nitrile by any of the techniques described hereinabove.

In the instances in which both \( R_1 \) and \( R_2 \) are the same \( C_1-C_3 \) primary alkyl, the desired N,N-disubstituted tyrosine can be prepared by the following sequence:

\[
\begin{align*}
\text{H}_2\text{N-}(\text{AA})\text{-COOH} & \xrightarrow{\text{R}_x\text{CHO}} \text{H}_2\text{, Pd/C} & \xrightarrow{\text{R}_x\text{CH_2}} \text{N-}(\text{AA})\text{-COOH} \\
\end{align*}
\]

In the foregoing, \( R_x\text{CHO} \) represents formaldehyde, acetaldehyde, or propionaldehyde.

In those instances in which \( R_1 \) and \( R_2 \) are different \( C_1-C_3 \) primary alkyl groups, the N,N-disubstituted tyrosine is available by treating the appropriate N-monosubstituted tyrosine, prepared in accordance with the foregoing sequence, with formaldehyde or acetaldehyde as described hereinabove.

In certain of the compounds of formula (I), the group \( R_5 \) is \( C_1-C_3 \) primary alkyl. In those instances, the appropriate \( \alpha \)-carbon substituted amino acid or its corresponding ester, amide, alcohol, or nitrile derivative is employed in the preparative sequence. The particular \( \alpha \)-carbon substituted phenylalanine can be prepared using the method described by Stein et al., *Journal of the American Chemical Society*, Vol. 77, 700-703 (1955). Resolution of the racemic mixture is effected in accordance with Turk et al., *J. Org. Chem.*, Vol. 40, No. 7, 953-955 (1975). The resulting \( \alpha \)-substituted phenyl-
alanine can be converted to the corresponding amide, alcohol, or nitrile in accordance with the methods described hereinabove. This can be carried out either before or after it has been used in preparation of the tetrapeptide sequence; however, it is highly preferred that it be accomplished prior to preparation of the tetrapeptide.

Those compounds of formula (I) in which Y is acetyl are prepared from the corresponding peptide in which Y is hydrogen and the terminal amino group is suitably blocked. This latter compound is treated with acetic anhydride in the presence of pyridine to produce the corresponding N-blocked, O-acetyl peptide. Upon deblocking with a mixture of hydrochloric acid and acetic acid, the desired compound is obtained.

The compounds of formula (I) are valuable pharmaceutical agents. They exhibit analgesic activity, and they especially are useful upon parenteral administration to mammals, including humans.

The compounds of formula (I) can be administered as such, or they can be compounded and formulated into pharmaceutical preparations in unit dosage form for parenteral administration. In the compounding or formulation, organic or inorganic solids and/or liquids which are pharmaceutically acceptable carriers can be employed. Suitable such carriers will be well recognized by those of ordinary skill in the art. The compositions may take the form of tablets, powder granules, capsules, suspensions, solutions, and the like.
The compounds of formula (I) when administered in an effective amount, will produce an analgesic effect. Dose levels may range generally from about 0.1 milligram to about 100 milligrams per kilogram body weight of the recipient. The preferred dose range generally is from about 1.0 milligram to about 20 milligrams per kilogram body weight of the recipient.

The following examples are provided to illustrate the preparation and activity of the compounds of formula (I). They are not intended to be limiting upon the scope thereof.

Example 1
Preparation of L-Tyrosyl-D-Alanyl-Glycyl-N\textsuperscript{\alpha}-Methyl-L-Phenylalanyl Amide, Acetate Salt.

A. Benzyl D-Alinate p-Toluenesulfonate.

To a mixture of 100 ml. of benzyl alcohol and 200 ml. of benzene containing 55.1 g. (0.29 mole) of p-toluenesulfonic acid monohydrate was added 25 g. (0.281 mole) of D-alanine. The mixture was brought to reflux, and water was removed azeotropically in a Dean-Stark apparatus. The mixture was heated for fifteen hours and then was cooled to room temperature and diluted with ether. The resulting precipitate was collected and recrystallized from methanol-ether to afford 55.3 g. (56%) of the title compound, m.p. 112-115°C.

Analysis, calculated for C\textsubscript{17}H\textsubscript{21}NO\textsubscript{5}S (351.42):

C, 58.10; H, 6.02; N, 3.99.

Found: C, 58.19; H, 6.06; N, 3.82.
B. Benzyl Nα-t-Butyloxy carbonyl-O-benzyl-L-tyrosyl-D-alinate.

To 200 ml. of dry N,N-dimethylformamide (DMF) was added 35.1 g. (0.1 mole) of the product from Part A. The resulting mixture was stirred and cooled to 0°C., and 11.2 g. (0.1 mole) of diazabicyclooctane (DABCO) was added. The mixture was stirred for ten minutes at 0°C., and 37.1 g. (0.1 mole) of Nα-t-butyloxy carbonyl-O-benzyl-L-tyrosine was added followed by 13.5 g. (0.1 mole) of 1-hydroxybenzotriazole (HBT) and 20.6 g. (0.1 mole) of N,N'-dicyclohexylcarbodiimide (DCC). The resulting mixture was stirred at 0°C. for three hours and then at room temperature for twenty-four hours. The mixture then was cooled to 0°C., the resulting suspension was filtered, and the filtrate was concentrated in vacuo. The resulting residue then was redissolved in ethyl acetate and was washed successively with 1N NaHCO₃, water, 0.75 N cold citric acid, and water. The organic layer then was dried over magnesium sulfate, filtered, and concentrated in vacuo. The resulting residue then was dissolved in hot ethanol. Crystallization ensued upon cooling. After one recrystallization from ethanol, 41.5 g. (80%) of pure title compound was obtained, m.p. 121-123°C.

Analysis, calculated for C₃₀H₃₆N₂O₆ (520.63):

C, 69.21; H, 6.97; N, 5.38.

Found: C, 68.99; H, 6.75; N, 5.17.
C. \( N^\alpha -t\)-Butyloxy carbonyl-O-benzyl-L-tyrosyl-D-alanine.

To a mixture of 200 ml. of tetrahydrofuran (THF) and 20 ml. of water was added 31.2 g. (0.06 mole) of the product from Part B. The resulting solution was cooled to 0°C., and 13.2 ml. (1.1 equiv.) of 5N sodium hydroxide was added slowly. The resulting mixture was stirred and allowed slowly to warm to room temperature. After five hours, the mixture was partitioned between water and ether. The aqueous layer was separated and cooled, the pH was adjusted to 2 by addition of citric acid, and the mixture was extracted with ethyl acetate. The ethyl acetate extract was washed with water, dried over magnesium sulfate, filtered, and diluted with ether. The resulting precipitate was collected to afford 17.7 g (67%) of the title compound, m.p. 160-162°C.

Analysis, calculated for \( C_{24}H_{30}N_2O_6 \) (442.51):

- C, 65.14; H, 6.83; N, 6.63.
- Found: C, 64.73; H, 6.70; N, 6.20.

D. Benzyl \( N^\alpha -t\)-Butyloxy carbonyl-O-benzyl-L-tyrosyl-D-alanyl-glycinate.

To 70 ml. of dry DMF was added 6.74 g. (0.02 mole) of the p-toluenesulfonic acid salt of benzyl glycinate. The resulting mixture was cooled to 0°C., and 2.24 g. (0.020 mole) of DABCO was added. The mixture was stirred for a few minutes, and 8.84 g. (0.020 mole) of the product of Part C was added followed by 2.7 g. (0.020 mole) of the HBT and 4.12 g. (0.020 mole) of DCC. The reaction mixture was stirred for two hours at 0°C. and then
for twenty-four hours at room temperature. The resulting suspension was cooled to 0°C., filtered, and the filtrate was concentrated \textit{in vacuo}. The resulting residue was dissolved in ethyl acetate and was washed successively with 1N sodium bicarbonate, water, cold 0.15 N citric acid, and water. The organic phase was dried over magnesium sulfate, filtered, and concentrated \textit{in vacuo}. The resulting residue was crystallized from ethanol to give 10.8 g. (92%) of pure title compound, m.p. 145-147°C.

Analysis, calculated for C$_{33}$H$_{39}$N$_3$O$_7$ (589.69):

- C, 67.22; H, 6.67; N, 7.13.
- Found: C, 67.32; H, 6.83; N, 6.91.

E. N$^\alpha$-t-Butyloxycarbonyl-O-benzyl-L-tyrosyl-D-alanyl-glycine.

To 150 ml. of a 9:1 mixture of tetrahydrofuran and water were added 15.95 gms. (27 mmoles) of the product from Part D. The mixture was cooled to 0°C. with stirring, and 30 ml. of 1N sodium hydroxide were added dropwise to the resulting mixture. The mixture was stirred for 2 hours upon completion of the dropwise addition and then was extracted twice with ether. The separated aqueous layer was acidified to pH 2.5 by addition of 30 ml. of 1N hydrochloric acid. The title compound crystallized, was collected by filtration, and was recrystallized once from a mixture of methanol and water and twice from ethyl acetate to give 11.43 gms. (85% theory), m.p. 104-107°C. $[\alpha]_D^{25}$ +31.4° (C = .5, MeOH).

Analysis, calculated for C$_{26}$H$_{33}$N$_3$O$_7$ (499.54):

- C, 62.51; H, 6.66; N, 8.41.
- Found: C, 62.31; H, 6.83; N, 8.12.
F. \( \text{N}^\alpha - \text{t-Butyloxy carbonyl-} \text{N}^\alpha \text{-methyl-L-phenyl alanine, d- (+) } \alpha \text{-methyl benzylamine salt.} \)

To 75 ml. of tetrahydrofuran were added 13.26 gms. (0.05 moles) of \( \text{N}^\alpha - \text{t-Butyloxy carbonyl-L-phenyl alanine.} \) The resulting mixture was added dropwise over a 30 minute period to a mechanically stirred suspension of 0.15 mole of potassium hydride and 0.5 gram of 18-crown-6 ether at 0°C. under a nitrogen atmosphere. The mixture was stirred for an additional hour at 0°C. A solution of 6.23 ml. (0.1 mole) of methyl iodide in 15 ml. of tetrahydrofuran was added dropwise over a 15 minute period. The mixture was maintained for two hours, and a mixture of 10 ml. of acetic acid and 10 ml. of tetrahydrofuran was added dropwise followed by 20 ml. of ethanol. The mixture then was poured onto 400 ml. of ice. The pH of the resulting aqueous phase then was raised to 12-13 by addition of 2N sodium hydroxide. The aqueous mixture was extracted twice with ether and then was acidified to pH 3.0 by addition of solid citric acid. The aqueous mixture then was extracted three times with 200 ml. of ether. The ether extracts were combined, extracted with water, dried over magnesium sulfate, and evaporated in vacuo to a syrup. The syrup was dissolved in 50 ml. of ether, and 6.44 ml. (0.05 moles) of d(+)-α-methyl benzylamine were added. The resulting solution was diluted with 350 ml. of hexane and was scratched. The product was collected by filtration to give 15.83 gms. (79% theory) of the title compound. Re-
crystallization from ethyl acetate gave 13.70 gms. (68% theory) of the title compound, m.p. 136-139°C. \([\alpha]_D^{25} -28.2^\circ \text{ (C = 1, EtOH)}\).

Analysis, Calculated for \(C_{23}H_{32}N_2O_4\) (400.50):

- C, 68.97; H, 8.05; N, 6.99.
- Found: C, 68.75; H, 7.81; N, 6.74.

G. \(N^\alpha\)-t-Butyloxycarbonyl-\(N^\alpha\)-methyl-L-phenylalanylamide.

\(N^\alpha\)-t-Butyloxycarbonyl-\(N^\alpha\)-methyl-L-phenylalanine (4.0 gms.; 0.01 moles; prepared by acidification of the \(d(+)\)-\(\alpha\)-methylbenzylamine salt and extraction into ether) was dissolved in 20 ml. of \(N,N\)-dimethylformamide (DMF). The mixture was cooled to \(-15^\circ\text{C.}\), and 1.56 ml. (0.012 moles) of isobutyl chloroformate were added followed by 1.32 ml. (0.012 moles) of \(N\)-methylmorpholine. The reaction mixture was stirred for 10 minutes at \(-15^\circ\text{C.}\), and anhydrous ammonia was bubbled into the reaction mixture for 1.5 hours. The resulting mixture was stirred for one hour at \(-15^\circ\text{C.}\), and the mixture then was poured into a vessel containing 200 ml. of ice. The aqueous solution was extracted with ethyl acetate. The organic layer was separated and washed successively with 1.5N citric acid, water, 1N sodium bicarbonate and water. The ethyl acetate solution then was dried over magnesium sulfate and evaporated \textit{in vacuo} to a syrup which was crystallized from a mixture of ether and petroleum ether to give 2.12 grams (76% theory) of the title compound, m.p. 91-92°C. \([\alpha]_D^{25} -111.2^\circ \text{ (C = .5, CHCl}_3\).
Analysis, Calculated for $\text{C}_{15}\text{H}_{22}\text{N}_{2}\text{O}_{3}$ (278.33):

C, 64.73; H, 7.97; N, 10.06.

Found: C, 64.95; H, 7.81; N, 9.79.

H. N$^\alpha$-t-Butyloxycarbonyl-O-benzyl-L-tyrosyl-D-alanyl-glycyl-N$^\alpha$-methyl-L-phenylalanyl amide.

To 20 ml. of freshly prepared glacial acetic acid containing anhydrous hydrogen chloride (1N) and 2 ml. of anisole were added 1.95 gms. (0.007 moles) of N$^\alpha$-t-butyloxycarbonyl-N$^\alpha$-methyl-L-phenylalanyl amide. The resulting mixture was stirred at room temperature for 30 minutes. The mixture then was poured into ether, and the resulting precipitate was collected and dried (1.5 gms.). The hydrochloride salt then was dissolved in 30 ml. of DMF. The solution was cooled to 0°C., and 1.4 ml. (0.007 moles) of dicyclohexylamine were added. The mixture was stirred for a few minutes, and 3.5 gms. (0.007 moles) of N$^\alpha$-t-butyloxycarbonyl-O-benzyl-L-tyrosyl-D-alanyl-glycine, 950 mg. (0.007 moles) of HBT, and 1.4 gms. (0.007 moles) of DCC were added. The reaction mixture then was stirred at 0°C. for 2 hours and then at 4°C. for 24 hours. The mixture was cooled to 0°C. and filtered. The filtrate was concentrated in vacuo to an oil which was redissolved in ethyl acetate. The ethyl acetate solution was extracted successively with 1N sodium bicarbonate, water, cold 0.75N citric acid, and water. The organic phase was dried over magnesium sulfate and concentrated in vacuo to an oil. The oil was chromatographed on a 40 cm. x 3 cm. column of Grace and Davison Grade 62 silica gel in chloroform. The product was eluted using a stepwise
gradient of chloroform to a mixture of 10% methanol in chloroform. The product was isolated according to the thin-layer profile of the fractions collected to give 3.55 gms. (77% theory) of the title compound. 

\[ \alpha \]_D^25 -9.2° (C = .5, MeOH).

Analysis, Calculated for C$_{36}$H$_{45}$N$_5$O$_7$ (659.8):

- C, 65.54; H, 6.57; N, 10.61.
- Found: C, 65.46; H, 6.58; N, 10.36.

I. N$^\alpha$-t-Butyloxy carbonyl-L-tyrosyl-D-alanyl-glycyl-N$^\alpha$-methyl-L-phenylalanyl amide.

The product from Part H (3.2 gms; 0.0485 moles) was dissolved in 60 ml. of ethanol, and 1.5 gms. of 5% palladium on carbon were added to the mixture as a water slurry. Nitrogen was bubbled into the reaction mixture through a gas dispersion tube for about 5 minutes followed by hydrogen gas for 6 hours. The reaction mixture then was flushed with nitrogen, and the palladium catalyst was removed by filtration. The mixture was concentrated in vacuo to a syrup. The syrup was dissolved in chloroform and absorbed onto a 40 cm. x 3 cm. chromatographic column containing Grace and Davison Grade 62 silica gel. The product was eluted using a stepwise gradient of chloroform to 10% methanol in chloroform and was isolated according to the thin-layer profile of the fractions collected to give 2.0 gms. (74% theory). \[ \alpha \]_D^25 -9.9° (C = .5, MeOH).

Amino acid analysis, Found: Gly, 1.01; Ala, 0.99; Tyr, 0.99; NH$_3$, 1.14.
J. L-Tyrosyl-D-alanyl-glycyl-N^α-methyl-L-phenylalanyl amide, acetate salt.

The product from Part I (1.6 gms.; 0.00281 moles) was dissolved in 10 ml. of trifluoroacetic acid containing 0.5 ml. of anisole. The mixture was stirred at 0°C. for 30 minutes. The mixture then was poured into ether, and the resulting precipitate was collected and dried (1.1 gms.). The solid was dissolved in sufficient aqueous buffer solution (1% pyridine and 0.05% acetic acid) to make 15 ml., and the solution was applied to a 2.5 cm. x 99 cm. column of DEAE-Sephadex A-25 (acetate) which had been equilibrated with the same buffer. The eluate was monitored at 280 nm, and the appropriate fractions were combined and lyophilized. Re-lyophilization from 10% acetic acid, followed by lyophilization from a 75:25 mixture of water and acetonitrile gave 0.84 gms. of the title compound. [α]D^25 +27.8° (C = 1, 1N HCl).

Example 2

Preparation of L-Tyrosyl-D-Alanyl-Glycyl-L-α-Methyl-phenylalanyl Aride, Acetate Salt.

A. L-α-Methylphenylalanine, benzyl ester, tosylate salt.

To 100 ml. of benzene were added 3.0 grams (0.0168 moles) of L-α-methylphenylalanine. To the resulting suspension then were added 3.5 grams (1.1 equiv.) of p-toluenesulfonic acid hydrate and 10 ml. of benzyl alcohol. The mixture was refluxed in the presence of a Dean-Stark water trap for four days. The mixture then was cooled to room temperature, and
ether was added to precipitate the tosylate salt. The resulting precipitate was collected and dried to give 7.0 grams (94%) of the title compound, m.p. 129-131°C. $[\alpha]_D^{25} -10.7^\circ$ (C = .5, 1N MeOH).

Analysis, Calculated for C$_{24}$H$_{27}$NO$_5$S (441.5):

N, 3.17.

Found: N, 2.87.

B. N$^\alpha$-t-Butyloxycarbonyl-O-benzyl-L-tyrosyl-D-alanyl-glycyl-L-$\alpha$-methylphenylalanine, benzyl ester.

To 80 ml. of DMF were added 5.74 grams (0.013 mmoles) of the product from Part A. The resulting mixture was cooled to 0°C. for 5 minutes, and 6.5 grams (13 mmoles) of N$^\alpha$-t-butyloxycarbonyl-O-benzyl-L-tyrosyl-D-alanyl-glycine (prepared as in Example 1), 1.8 grams (13 mmoles) of HBT, and 2.7 grams (13 mmoles) of DCC were added. The mixture was stirred at 0°C. for two hours and then at room temperature for 24 hours. The mixture then was cooled to 0°C., and the resulting precipitate was removed by filtration. The filtrate was evaporated in vacuo. The resulting residue was dissolved in ethyl acetate, and the ethyl acetate solution was extracted successively with 1N sodium bicarbonate, water, 0.75N citric acid, and water. The organic phase then was dried over magnesium sulfate and evaporated in vacuo to an oil. The oil was crystallized from ether and recrystallized from a mixture of ethyl acetate and ether to give 7.0 gram (72%) of the title compound. $[\alpha]_D^{25} +7.9^\circ$ (C = .5, MeOH).
Analysis, Calculated for $\text{C}_4\text{H}_5\text{O}_8\text{N}_4$ (750.86):  
C, 68.78; H, 6.71; N, 7.46.

Found: C, 68.75; H, 6.46; N, 7.21.

C. $\text{N}^\alpha$-$t$-Butyloxy carbonyl-$L$-tyrosyl-$D$-alanyl-glycyl-$L$-$\alpha$-methylphenylalanine, dicyclohexylamine salt.

To 50 ml. of ethanol were added 4.0 grams (0.0053 moles) of the product from Part B. A slurry of 2.0 grams of 5% palladium-on-carbon in DMF then was added. Nitrogen was bubbled into the mixture through a gas dispersion tube for 5 minutes followed by hydrogen gas for 4 hours. The mixture then was flushed with nitrogen, and the palladium catalyst was removed by filtration. The filtrate was concentrated in vacuo to a syrup. The syrup in chloroform was applied to a 10 cm. x 2 cm. column containing Grace and Davison Grade 62 silica gel. The column was eluted with a step gradient of chloroform + chloroform-methanol (9.5:0.5). The major fractions were combined, and the solvent was evaporated. The resulting oil was dissolved in ethyl acetate, and 1 ml. of dicyclohexylamine was added. The resulting precipitate was collected and dried to give 2.6 grams (65%) of the title compound, m.p. 142-146°C. $\left[a\right]_{D}^{25} +46.3^\circ$ (C = .5, MeOH).

D. $\text{N}^\alpha$-$t$-Butyloxy carbonyl-$L$-tyrosyl-$D$-alanyl-glycyl-$L$-$\alpha$-methylphenylalanine amide.

The product from Part C (2.0 grams; 0.0027 moles) was neutralized with a mixture of ethyl acetate and 0.75N citric acid. The resulting organic layer was separated, extracted with water,
dried over magnesium sulfate, and evaporated in vacuo to an oil (1.5 grams). The resulting free acid was dissolved in 30 ml. of DMF, and the solution was cooled to 0°C. in a pressure bottle. DCC (560 mg.; 0.0027 moles) was added, and the mixture was stirred for 4 hours at 0°C. and then for 3 hours at room temperature. The bottle then was cooled to -78°C., and 30 ml. of anhydrous ammonia were added. The bottle was again sealed, and the mixture was allowed to stir at room temperature for 48 hours. The mixture was cooled to -78°C., the bottle was opened, and ammonia was allowed to evaporate at room temperature. The solvent then was evaporated in vacuo. The resulting residue was dissolved in ethyl acetate, and the ethyl acetate solution was extracted first with 0.75 N citric acid and then with water. The solution was dried over magnesium sulfate, and the solvent was evaporated in vacuo. The residue was dissolved in chloroform and applied to a 3 cm. x 45 cm. column of Grace and Davison Grade 62 silica gel. The column was eluted with a step gradient comprising chloroform + chloroform:methanol (9:1). Fractions were combined on the basis of the TLC profile to give, after evaporation of solvent, 1.1 grams (72%) of the title compound. [α]_D^25 -26° (C = .4, 'MeOH).

Amino acid analysis, Found: Gly, 0.99; Ala, 1.00; Tyr, 0.99; NH₃, 1.12.

E. L-Tyrosyl-D-alanyl-glycyl-L-α-methyl-phenylalanyl amide, acetate salt.

To 20 ml. of a mixture of 1N gaseous hydrogen chloride in glacial acetic acid and con-
taining 0.3 ml. of anisole were added 900 mg.
(0.0016 moles) of the product from Part D. The
mixture was stirred at room temperature for 30
minutes and then was poured into ether. The re-
sulting precipitate was collected and dried (720
mg.). The solid was dissolved in sufficient aqueous
buffer solution (1% pyridine and 0.05% acetic acid)
to achieve 5 ml. volume, and the solution was
applied to a 2.5 cm. x 99 cm. column of DEAE-
Sephadex A-25 (acetate) previously equilibrated with
the same buffer. The eluate was monitored at
280 nm, and appropriate fractions were combined and
lyophilized. Re-lyophilization from 10% acetic acid
followed by lyophilization from a 75:25 mixture of
water and acetonitrile gave 400 mg. of the title
compound. [α]D25  +23.9° (C = 0.5, 1N HCl).
Analysis, Calculated for C26H35N5O7 (529.60):
C, 58.97; H, 6.66; N, 13.22; O, 21.15.
Found: C, 59.02; H, 6.36; N, 12.99; O, 21.41.
Amino acid analysis, Found: Tyr, 0.96; Ala,
1.01; Gly, 1.06; NH3, 1.03.

Example 3
Preparation of L-Tyrosyl-D-Alanyl-Glycyl-Nα-n-
Propyl-L-Phenylalanyl Amide, Acetate Salt.
A. Nα-t-Butyloxycarbonyl-Nα-n-propyl-
L-phenylalanine.
To 70 ml. of tetrahydrofuran were added
10.6 gms. (0.04 moles) of Nα-t-butyloxycarbonyl-L-
phenylalanine. The resulting mixture was added
dropwise over a 30 minute period to a mechanically
stirred suspension of 0.12 mole of potassium hydride
in 220 ml. of tetrahydrofuran and 0.5 gm. of 18-
crown-6 ether at 0°C. under a nitrogen atmosphere. The mixture was stirred for an additional 10 min. at 0°C. A solution of 23.3 ml. (0.24 mole) of 1-iodo-propane in 40 ml. of tetrahydrofuran was added dropwise over a 20 minute period. The mixture was maintained for 2.5 hours at 0°C., and another 11.5 ml. (0.12 mole) of 1-iodopropane was added dropwise to the mixture. The mixture was stirred an additional 2 hours at 0°C., 10 ml. of glacial acetic acid was added, and the mixture stirred for 10 minutes. The mixture then was poured onto crushed ice. The pH of the resulting aqueous phase then was raised to 8.0 with 2N sodium hydroxide. The aqueous mixture was extracted twice with ether, and then was acidified to pH 2.5 by addition of cold 2N HCl. The aqueous mixture then was extracted with ethyl acetate. The ethyl acetate extract was extracted once with water, dried (over MgSO₄), and evaporated in vacuo to a syrup. The syrup was dissolved in 200 ml. of ether, and 8 ml. (0.04 mole) of DCHA was added. The precipitate was filtered and the filtrate was extracted once with 1.5N citric acid, and water. The ether layer was dried (over MgSO₄) and evaporated in vacuo to a oil. The oil was chromatographed on a 40 cm. x 3 cm. column of Grace and Davison Grade 62 silica gel in chloroform. The product was eluted using a stepwise gradient of chloroform to a mixture of 5% methanol in chloroform. The product was isolated according to the thin-layer profile of the fractions collected to give 3.6 gms. (31% theory) of the title compound. \([\alpha]_{D}^{25} = -153.3 \ (C = 1, \text{MeOH})\). NMR \(\delta (-\text{CO}_2\text{H}) = 10.47; \ \delta (\text{Me}_3\text{C}^-) = 1.50.\)
Analysis, Calculated for C_{16}H_{25}NO_{4} (295.4):
C, 65.06; H, 8.53; N, 4.74.

Found: C, 65.26; H, 8.29; N, 4.69.

B. N^\alpha-t-Butyloxy carbonyl-N^\alpha-n-propyl-L-phenylalanyl amide.

The N^\alpha-t-butyloxycarbonyl-N^\alpha-n-propyl-L-phenylalanine (prepared in part A) is dissolved in N,N-dimethylformamide (DMF). The mixture was cooled to -15°C, and one equivalent of isobutyl chloroformate was added followed by one equivalent of N-methylmorpholine. The mixture was stirred for 10 minutes at -15°C, and anhydrous ammonia was bubbled into the mixture for 30 minutes. The resulting mixture was stirred for one hour at -15°C, and the mixture then was poured into a vessel containing 200 ml. of ice. The aqueous solution was extracted with ethyl acetate. The organic layer was separated and washed successively with 1.5N citric acid, water, 1N sodium bicarbonate and water. The ethyl acetate solution then was dried (over MgSO_4) and evaporated in vacuo to yield the title compound.

C. N^\alpha-t-Butyloxy carbonyl-L-tyrosyl-D-alanyl-glycyl-N^\alpha-n-propyl-L-phenylalanyl amide.

To 20 ml. of freshly prepared glacial acetic acid containing anhydrous hydrogen chloride (1N) and 2 ml of anisole was added one equivalent of N^\alpha-t-butyloxycarbonyl-N^\alpha-n-propyl-L-phenylalanyl amide. The resulting mixture was stirred at room temperature for 30 minutes. The mixture then was poured into ether, and the resulting precipitate was collected and dried. The hydrochloride salt then was dissolved in 30 ml. of DMF. The solution was
cooled to 0°C., and one equivalent of dicyclohexyl-
amine was added. The mixture was stirred for a few
minutes, and one equivalent of N-tert-butyloxy carbonyl-
O-benzyl-L-tyrosyl-D-alanyl-glycine (prepared as in
Example 1E), one equivalent of HBT, and one equivalent
of DCC were added. The reaction mixture then was
stirred at 0°C. for 2 hours and then at 4°C. for 24
hours. The mixture was cooled to 0°C. and filtered.
The filtrate was concentrated in vacuo to an oil
which was redissolved in ethyl acetate. The ethyl
acetate solution was extracted successively with 1N
sodium bicarbonate, water, cold 0.75N citric acid,
and water. The organic phase was dried over magnesium
sulfate and concentrated in vacuo to an oil. The
oil was chromatographed on a 40 cm. x 3 cm. column
of Grace and Davison Grade 62 silica gel in chloroform.
The product was eluted using a stepwise gradient of
chloroform to a mixture of 10% methanol in chloroform.
The product was isolated according to the thin-layer
profile of the fractions collected to give N-tert-
butyloxy carbonyl-O-benzyl-L-tyrosyl-D-alanyl-glycyl-
N-Propyl-L-phenylalanyl amide.

The product from the above paragraph was
dissolved in 60 ml. of ethanol, and 1.5 gms. of 5%
palladium-on-carbon were added to the mixture as a
water slurry. Nitrogen was bubbled into the reaction
mixture through a gas dispersion tube for about 5
minutes followed by hydrogen gas for 6 hours. The
reaction mixture then was flushed with nitrogen, and
the palladium catalyst was removed by filtration.
The mixture was concentrated in vacuo to a syrup.
The syrup was dissolved in chloroform and absorbed
onto a 40 cm. x 3 cm. chromatographic column containing Grace and Davison Grade 62 silica gel. The product was eluted using a stepwise gradient of chloroform to 10% methanol in chloroform and was isolated according to the thin-layer profile of the fractions collected to yield the title compound. 

\[ \alpha_D^{25} -34.8^\circ \text{ (C = .5, MeOH).} \]

Analysis, Calculated for C$_{31}$H$_{43}$N$_5$O$_7$ (597.7):

- C, 62.29; H, 7.25; N, 11.72.
- Found: C, 62.13; H, 7.24; N, 11.70.

D. L-Tyrosyl-D-aleryl-glycyl-$N^\alpha$-n-propyl-L-phenylalanyl amide, acetate salt.

The product from Part C (800 mg.; 1.34 mmoles) was dissolved in 10 ml. of trifluoroacetic acid containing 0.5 ml. of anisole. The mixture was stirred at 0°C. for 30 minutes. The reaction mixture was lyophilized. The solid was dissolved in sufficient aqueous buffer solution (1% pyridine and 0.05% acetic acid) to make 10 ml., and the solution was applied to a 2.5 cm. x 99 cm. column of DEAE-Sephadex A-25 (acetate-\(\alpha\)) which had been equilibrated with the same buffer. The eluate was monitored at 280 nm, and the appropriate fractions were combined and lyophilized. Re-lyophilization from 1M acetic acid gave 655 mg. of the title compound. 

\[ \alpha_D^{25} -11.0^\circ \text{ (C = .5, 1N HCl).} \]

Analysis, Calculated for C$_{28}$H$_{39}$N$_5$O$_7$ (557.6):

- C, 60.31; H, 7.05; N, 12.56.
- Found: C, 60.23; H, 6.98; N, 12.49.

Amino acid analysis, Found: Tyr, 0.99; Ala, 1.00; Gly, 1.01; NH$_3$, 0.96.
Example 4

Preparation of L-Tyrosyl-D-Alanyl-Glycyl-\(N^\alpha\)-Ethyl-L-Phenylalanyl Amide, Acetate Salt.

A. \(N^\alpha\)-Butyloxycarbonyl-\(N^\alpha\)-ethyl-L-phenylalanine.

To 70 ml. of tetrahydrofuran were added 10.6 gms. (0.04 mole) of \(N^\alpha\)-butyloxycarbonyl-L-phenylalanine. The resulting mixture was added dropwise over a 30 minute period to a mechanically stirred suspension of 0.12 mole of potassium hydride in 220 ml. of tetrahydrofuran and 0.5 gm. of 18-crown-6 ether at 0°C. under a nitrogen atmosphere. The mixture was stirred for an additional 10 minutes at 0°C. A solution of 19.4 ml. (0.24 mole) of ethyl iodide in 40 ml. of tetrahydrofuran was added dropwise over a 20 minute period. The mixture was maintained for four hours at 0°C. Another 19.4 ml. (0.24 mole) of ethyl iodide was added in two equal portions to the mixture. The mixture was stirred for an additional two hours at 0°C., and then 10 ml. of glacial acetic acid was added. After stirring the mixture for 10 minutes, the mixture was poured onto 400 ml. of crushed ice. The pH of the resulting aqueous phase was raised to pH 8.0 by addition of 2N sodium hydroxide. The aqueous mixture was extracted twice with ether and then was acidified to pH 2.5 by addition of cold 2N hydrochloric acid. The aqueous mixture then was extracted with ethyl acetate. The extract was washed with water, dried (over \(\text{MgSO}_4\)), and evaporated in vacuo to a syrup. The syrup was dissolved in 200 ml. of ether, and 8 ml. (0.04 mole) of DCHA was added. The precipitate was filtered,
and the filtrate was extracted with 1.5N citric acid, and water. The ether layer was dried (over MgSO₄) and evaporated in vacuo to give 4.6 gms. (39% theory) of the title compound. NMR δ (phenyl) = 7.2; δ (Me₃COC-) = 1.4.

B. Nα-t-Butyloxycarbonyl-Nα-ethyl-L-phenylalanyl amide.

Nα-t-Butyloxycarbonyl-Nα-ethyl-L-phenylalanine (4.3 gms; 0.0146 mole; prepared in Part A) was dissolved in 60 ml. of N,N-dimethylformamide (DMF). The mixture was cooled to 0°C., and 3.0 gms (0.0146 mole) of N,N'-dicyclohexylcarbodiimide (DCC) was added. The reaction mixture was stirred for two hours at 0°C. and then 72 hours at room temperature. The mixture then was cooled to 0°C. and filtered. The filtrate was concentrated in vacuo to an oil which was redissolved in ethyl acetate. The solution was extracted with 1N sodium bicarbonate, water, cold 1.5N citric acid, and water. The organic phase was dried (over MgSO₄) and concentrated to give 3.93 gms. (91% theory) of the title compound. [α]₂⁵_D -101.51° (C = 1, MeOH).

Analysis, Calculated for C₁₆H₂₄N₂O₃ (292.4):

C, 65.73; H, 8.27; N, 9.58.

Found: C, 66.03; H, 8.13; N, 9.85.
C. N\(^\alpha\)-Ethyl-L-phenylalanyl amide, hydrochloride salt.

N\(\alpha\)-t-Butyloxycarbonyl-N\(\alpha\)-ethyl-L-phenylalanyl amide (3.5 gms.; 11.95 mmoles; prepared in Part B) was dissolved in 40 ml. of freshly prepared glacial acetic acid containing anhydrous hydrogen chloride (1N) and 1.5 ml. of anisole, and 1.5 ml. of \((\text{C}_2\text{H}_5)_3\text{SiH}\). The resulting mixture was stirred at room temperature for 30 minutes. The mixture then was poured into ether, and the resulting precipitate was collected and dried to give 2.6 gms. (96% theory) of the title compound, m.p. 276-277°C.

Analysis, Calculated for C\(_{11}\)H\(_{16}\)N\(_2\)Cl (227.7):

\[\text{C, 58.02; H, 7.08; N, 12.30.}\]

\[\text{Found: C, 57.97; H, 7.26; N, 12.54.}\]

D. N\(\alpha\)-t-Butyloxycarbonyl-L-tyrosyl-D-alanyl-glycyl-N\(\alpha\)-ethyl-L-phenylalanyl amide.

To 50 ml. of DMF were added 1.14 gms. (0.005 mole) of N\(\alpha\)-ethyl-L-phenylalanyl amide, hydrochloride salt, (prepared in Part C). The mixture was cooled to 0°C. and then 2.95 gms. (0.005 mole) of N\(\alpha\)-t-butyloxycarbonyl-L-tyrosyl-D-alanyl-glycine, DCHA salt, was added. The mixture was stirred at 0°C. for 5 minutes, and then 675 mg. (0.005 mole) of iET and 1.03 gms. (0.005 mole) of DCC were added. The reaction mixture was stirred at 0°C. for 6.5 hours and then at room temperature for 20 hours. The mixture was cooled to 0°C. and filtered. The filtrate was concentrated \textit{in vacuo} to an oil which was redissolved in ethyl acetate, extracted with 1N sodium bicarbonate, water, cold 1.5N citric acid, and water. The organic phase was dried (over
MgSO$_4$) and concentrated in vacuo to an oil. The oil was chromatographed on a 40 cm. x 3 cm. column of Grace and Davison Grade 62 silica gel in chloroform. The product was eluted using a stepwise gradient of chloroform to a mixture of 15% methanol in chloroform. The product was isolated according to the thin-layer profile of the fractions collected to give 1.13 gms (39% theory) of the title compound. $[\alpha]_D^{25} = -21.0^\circ$ (C = 0.5, MeOH).

Analysis, Calculated for C$_{30}$H$_{41}$N$_7$O$_7$ (583.7):

C, 61.73; H, 7.08; N, 12.00.

Found: C, 60.35; H, 7.26; N, 11.25.

E. L-Tyrosyl-D-alanyl-glycyl-$N^\alpha$-ethyl-L-phenylalanyl amide, acetate salt.

1.5 g product from Part D (1.71 mmoles) was dissolved in 20 ml. of trifluoroacetic acid containing 3 ml. of anisole and 3 ml. of ($C_2H_5)_3SiH$. The mixture was stirred at 0°C. for 30 minutes. The mixture then was poured into ether, and the resulting precipitate was collected and dried (660 mg.). The solid was dissolved in sufficient aqueous buffer solution (1% pyridine and 0.05% acetic acid) to make 10 ml., and the solution was applied to a 2.5 cm. x 90 cm. column of DEAE-Sephadex A-25 (acetate) which had been equilibrated with the same buffer. The eluate was monitored at 280 nm., and the appropriate fractions were combined and lyophilized. The solid was dissolved in 0.2M acetic acid (10 ml.) and the solution was chromatographed on a 2.5 cm. x 99 cm. column of G-10 Sephadex which had been equilibrated with the same solvent. The eluate was monitored at 280 nm., and the appropriate
fractions were combined and lyophilized to give 448 mg. (48% theory) of the title compound. [$\alpha$]$_D^{25}$ -10.6° (C = .5, 1N HCl).

Analysis, Calculated for C$_{27}$H$_{37}$N$_5$O$_7$ (543.6)

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<td>H</td>
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<td>N</td>
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</table>

Amino acid analysis, Found: Tyr, 1.03; Ala, 0.99; Gly, 0.97; NH$_3$, 0.98.

The compounds of formula (I) are useful as analgesics. The analgesic activity of the compounds of formula (I) is demonstrated by the mouse hot plate test. In this test, a mouse is placed inside an upright acrylic cylinder comprising, as its base, a hot plate surface which is maintained at 52°C. In this test, the mouse is given, by subcutaneous injection, a predetermined amount of test compound dissolved or suspended in a suitable carrier. A predetermined period subsequent to administration of the test compound is permitted to elapse, and the mouse then is placed on the hot plate surface. The latencies in seconds until the occurrence of each of two separate phenomena then are recorded. First, the latency until the mouse licks its hind paw is measured, and, secondly, the latency until the mouse jumps from the hot plate surface is measured. An agent which exhibits analgesic activity produces an increase in these latencies over those of control mice which receive injections only of the carrier. This must occur in a dose range which produces no motor incoordination or incapacitation. The following Tables record the results obtained from this test, comparing them with a saline control. Table I
provides latency to hind paw lick, and Table II provides latency to escape jump. The criterion for an affirmative analgesic effect is as follows: the latency for the hind paw lick or escape jump for a treated animal must be equal to or greater than the mean control latency plus two standard deviations of the mean. Each result provided in the following Tables I and II represents the mean value plus or minus standard error.
**Table I**

**Analgesic Activity**

Latency to Hind Paw Lick, Seconds

<table>
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<tr>
<th>Time Elapse, min.</th>
<th>Compound</th>
<th>Control</th>
<th>Dose, mg/kg.&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Time Elapse, min.</td>
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<td>Control</td>
<td>Dose, mg/kg. a</td>
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Table I (continued)

**Analgesic Activity**

*Latency to Hind Paw Lick, Seconds*

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### Table I (continued)

#### Analgesic Activity

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## Table II

**Analgesic Activity**

**Latency to Escape Jump, Seconds**

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</table>
Table II

Analgesic Activity

Latency to Escape Jump, Seconds

<table>
<thead>
<tr>
<th>Time Elapse, min.</th>
<th>Control</th>
<th>Dose, mg/kg. a</th>
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<tr>
<td>60</td>
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</table>
Table II (continued)

Analgesic Activity

Latency to Escape Jump, Seconds

<table>
<thead>
<tr>
<th>Time Elapse, min.</th>
<th>Compound</th>
<th>Control</th>
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<th>0.6</th>
<th>1</th>
<th>3</th>
<th>10</th>
<th>30</th>
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</tr>
</tbody>
</table>
Table II (continued)

Analgesic Activity

Latency to Escape Jump, Seconds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time Elapse, min.</th>
<th>Control</th>
<th>0.3 mg/kg.</th>
<th>1 mg/kg.</th>
<th>3 mg/kg.</th>
<th>10 mg/kg.</th>
<th>50 mg/kg.</th>
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<tr>
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<td>D</td>
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<td>185.1±15.0</td>
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<td>-</td>
<td>218.4±16.2</td>
<td>240.0±0.1</td>
<td>-</td>
</tr>
</tbody>
</table>
Footnotes:

a. The numerals "1", "2", and "3" appearing as superscripts indicate that the result is significant to P<0.001, to P<0.01, and to P<0.05, respectively.

b. The designations refer to the following compounds:

A. L-Tyrosyl-D-alanyl-glycyl-N^a-methyl-L-phenylalananyl amide, acetate salt.
B. L-Tyrosyl-D-alanyl-glycyl-L-a-methyl-phenylalananyl amide, acetate salt.
C. L-Tyrosyl-D-alanyl-glycyl-N^a-n-propyl-L-phenylalananyl amide, acetate salt.
D. L-Tyrosyl-D-alanyl-glycyl-N^a-ethyl-L-phenylalananyl amide, acetate salt.
The claims defining the invention are as follows:

1. A compound of the general formula

![Chemical Structure]

and pharmaceutically acceptable non-toxic acid addition salts thereof, in which L and D, when applicable, define the chirality;

- $R_1$ and $R_2$ independently are hydrogen or C$_1$-C$_3$ primary alkyl;
- $R_3$ is C$_1$-C$_4$ primary or secondary alkyl or -CH$_2$CH$_2$S-CH$_3$;
- $R_4$ is hydrogen or C$_1$-C$_3$ primary alkyl;
- $R_5$ is hydrogen or C$_1$-C$_3$ primary alkyl;
- $Y$ is hydrogen or acetyl; and
- $Z$ is -C-NH$_2$, -CH$_2$OH, or -CN; subject to the limitation that one of $R_4$ and $R_5$ is C$_1$-C$_3$ primary alkyl and the other is hydrogen.

2. A compound of claim 1, in which $Y$ is hydrogen.

3. A compound of claim 1, in which $R_1$ and $R_2$ are hydrogen.
4. A compound of claim 1, in which \( R_3 \) is methyl.

5. A compound of claim 1, in which \( R_4 \) is \( \text{C}_1-\text{C}_3 \) primary alkyl.

6. A compound of claim 5, in which \( R_4 \) is methyl.

7. A compound of claim 1, in which \( R_5 \) is \( \text{C}_1-\text{C}_3 \) primary alkyl.

8. A compound of claim 7, in which \( R_5 \) is methyl.

9. A compound of claim 1, in which \( Z \) is \(-\text{C}-\text{NH}_2\).

10. L-Tyrosyl-D-alanyl-glycyl-N\(^\alpha\)-methyl-L-phenylalanyl amide, acetate salt.

11. L-Tyrosyl-D-alanyl-glycyl-L-\( \alpha \)-methyl-phenylalanyl amide, acetate salt.

12. L-Tyrosyl-D-alanyl-glycyl-N\(^\alpha\)-n-propyl-L-phenylalanyl amide, acetate salt.

13. L-Tyrosyl-D-alanyl-glycyl-N\(^\alpha\)-ethyl-L-phenylalanyl amide, acetate salt.

14. A process for preparing a compound of the general formula
and pharmaceutically acceptable non-toxic acid addition salts thereof, in which L and D, when applicable, define the chirality;

15 \( R_1 \) and \( R_2 \) independently are hydrogen or \( C_1-C_3 \) primary alkyl;

\( R_3 \) is \( C_1-C_4 \) primary or secondary alkyl or \(-\text{CH}_2\text{CH}_2\text{-S-CH}_3\);

\( R_4 \) is hydrogen or \( C_1-C_3 \) primary alkyl;

\( R_5 \) is hydrogen or \( C_1-C_3 \) primary alkyl;

\( Y \) is hydrogen or acetyl; and

\( Z \) is \(-\text{C-NH}_2\), \(-\text{CH}_2\text{OH}\), or \(-\text{CN}\); subject to the limitation that one of \( R_4 \) and \( R_5 \) is \( C_1-C_3 \) primary alkyl and the other is hydrogen; which comprises cleaving the blocking agents from the correspondingly protected compound of formula (I) with an acid medium.
15. A pharmaceutical composition comprising and excipient and as active ingredient a compound of the general formula

\[
\begin{align*}
\text{N-CH-C-NH-CH-C-NH-CH-C-N-C-Z} \\
\text{R}_1 \quad \text{R}_2 \\
\text{R}_3 \quad \text{R}_4 \quad \text{R}_5 \\
\end{align*}
\]

and pharmaceutically acceptable non-toxic acid addition salts thereof, in which \( L \) and \( D \), when applicable, define the chirality;

- \( R_1 \) and \( R_2 \) independently are hydrogen or \( C_1-C_3 \) primary alkyl;
- \( R_3 \) is \( C_1-C_4 \) primary or secondary alkyl or \( -\text{CH}_2\text{CH}_2\text{-S-CH}_3 \);
- \( R_4 \) is hydrogen or \( C_1-C_3 \) primary alkyl;
- \( R_5 \) is hydrogen or \( C_1-C_3 \) primary alkyl;
- \( Y \) is hydrogen or acetyl; and
- \( Z \) is \( -\text{C-NH}_2, \ -\text{CH}_2\text{OH}, \) or \( -\text{CN} \); subject to the limitation that one of \( R_4 \) and \( R_5 \) is \( C_1-C_3 \) primary alkyl and the other is hydrogen.
16. A compound as claimed in claim 1 substantially as hereinbefore described with particular reference to any one of the Examples.

17. A process as claimed in claim 14 substantially as hereinbefore described with particular reference to any one of the Examples.

18. A pharmaceutical composition as claimed in claim 15 substantially as hereinbefore described.

DATED this FOURTEENTH day of SEPTEMBER, 1978

ELI LILLY AND COMPANY

Patent Attorneys for the Applicant
SPRUSON & FERGUSON